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TALLOW AND MEAT AND BONE MEAL AS
CHOLESTEROL SOURCES FOR RAINBOW TROUT
FEEDS

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FINAL REPORT

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**Tallow and Meat and Bone Meal as Cholesterol Sources for
Rainbow Trout Feeds**

**Final Report
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1. Summary

The replacement of fish meal and oil with plant protein and oil sources results in decreased growth performance in salmonid fish. One of the reasons for this may be that salmonids require nutrients present in fish products that are lacking in plant products. One of these nutrients may be cholesterol. To test this, a series of 3 experiments were performed. In experiment 1 we examined whether rainbow trout have a requirement for dietary cholesterol and the effect of dietary polyunsaturated omega-3 fatty acids (PUFA) on this requirement. There were 8 experimental diets arranged in a 2 x 4 factorial design with 2 levels of PUFA (olive oil or olive oil + linseed oil) and 4 levels of cholesterol (0.00, 0.05, 0.10 and 0.15%) added using synthetic cholesterol. Fish were weighed on day 0, 28, 56, and 84. The effect of PUFA was significant with fish on the olive oil diets having significantly lower total gain, feed intake, final body weight, TGC and significantly higher mortality ($P < 0.05$). Fish fed the 0.15% cholesterol diets ate significantly more than those on the 0% cholesterol diets. Although there were no other significant effects of cholesterol on growth parameters, the addition of cholesterol did result in increased total gain ($P = 0.073$). Unfortunately, analysis of the diets used showed that one of the plant ingredients used contained cholesterol. The experiment was therefore duplicated using diets containing no cholesterol. In Experiment 2 the effect of PUFA was not significant on any of the growth parameters measured. The addition of cholesterol to the diet significantly increased average daily gain and decreased feed:gain ratio ($P < 0.05$). This conclusively shows that fish have a requirement for cholesterol. Experiment 3 was performed to determine the effect of adding cholesterol to rainbow trout diets using tallow or meat and bone meal (MBM). There were 3 experimental diets including a negative control containing no cholesterol, a diet containing 4% tallow and a diet containing 10% MBM. The fish fed the tallow diet had significantly higher ADGs and lower Feed:Gain ratios than those fed the negative control diet ($P < 0.05$). Average daily feed intake was not significantly affected. The fish fed the MBM diets had ADGs that were significantly higher than those for the negative controls and tallow fed fish ($P < 0.05$). This indicates that while the addition of cholesterol alone using tallow to rainbow trout diets improves growth and feed conversion, MBM may contain other pronutritional factors that improve the growth of fish. More work is required to determine the identity of these factors. In conclusion, rainbow trout have a requirement for cholesterol to achieve maximal growth rates. Tallow and MBM are good sources of cholesterol and significantly improve the growth of rainbow trout fed plant-based diets.

Introduction

Historically fish meal and oil have been the most important sources of protein and energy in commercial feeds for finfish. This is due to several factors including, nutrient quality, nutrient density, palatability, low cost and abundance. Given the advantages that marine products possess, particularly for carnivorous fish, a compelling reason is needed to give the aquafeed industry an incentive to decrease its use. That reason of course is that the low cost and abundance of fish meal appears to be at an end. The rapid expansion of aquaculture production will require lowering the inclusion rate of fish meal and oil in aquafeeds and replacing them with non-marine protein and oil sources.

Current research at the University of Saskatchewan has concentrated on the use of plant protein and oil sources to replace fish meal and oil. We have performed extensive studies on the use of canola, peas, faba beans and flax in the last 4 years (Thiessen et al., 1993; Thiessen et al., 1994; Drew et al., 2005; Borgeson, 2005). While there has been significant progress in understanding the nutritional properties of these products in finfish, the replacement of fish meal and oil with plant ingredients still present problems in practical diet formulation.

On the oil side, the composition of vegetable oils is significantly different from that of fish oils. Fish oil is rich in polyunsaturated fatty acids (PUFA) including the n-3 fatty acids eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). In contrast, most vegetable oils do not contain significant amounts of fatty acids with chain lengths greater than 18 and most are relatively low in n-3 fatty acids. Exceptions to this are canola oil, with approximately 12% alpha linolenic acid (18:3n-3) and linseed oil, which contains approximately 53% 18:3n-3 (NRC, 1994). However, fish oil also contains other constituents which may act as growth factors in salmonid fish. While these growth factors have not been conclusively identified, cholesterol appears to be a likely candidate.

Fish oil contains 4-10 mg/g of cholesterol and fish meal contains significant levels of cholesterol depending on the source of the meal. In contrast, vegetable oils and proteins are devoid of cholesterol. The result is that in diets where a high proportion of the marine products have been replaced by vegetable ingredients, there may be extremely low levels of cholesterol present.

While teleost fish have the ability to synthesize cholesterol a recent study suggests that the rate of synthesis may not be sufficient to support maximal growth. Twibell and Wilson (2004) fed channel catfish a control diet containing 0.5% cod liver oil as the only source of cholesterol or the control diet supplemented with 1% cholesterol. The cholesterol-supplemented fish grew significantly faster and ate significantly more than the controls ($P < 0.05$). These results suggest that the low levels of cholesterol in diets with low or zero levels of marine ingredients may contribute to the growth depression observed when these diets are fed to salmonids.

In support of this notion, previous studies have shown that dietary PUFA can regulate gene expression in vertebrates. Fish oil feeding significantly affected the mRNA levels for lipogenic enzymes including hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in the cholesterol synthetic pathway (Horton et al., 2003). In another study, dietary oils rich in PUFA, including fish oil and vegetable oils, were shown to reduce the activity of HMG-CoA reductase in mice (Le Jossic-Corcus et al., 2005). However, fish oil and other marine byproducts also contain cholesterol so the decrease in cholesterol synthesis due to fish oil is balanced by the cholesterol it provides in the diet. In contrast, when vegetable oils rich in PUFA are fed, cholesterol synthesis is decreased but since vegetable oils do not contain cholesterol, a deficiency of this essential molecule may develop and limit the growth of fish. This problem is exacerbated by the replacement of fish meal, which is also a rich source of cholesterol, by vegetable protein sources. Alternatives to marine sources of cholesterol in salmonid diets are animal fats

and protein meals. Tallow is an extremely rich source of cholesterol providing approximately 1.0% cholesterol. Meat bone meal contains 0.14% cholesterol and in addition contains a approximately 50% protein with a relatively good amino acid balance and high digestibility (Bureau et al., 1999).

Surprisingly, the effect of replacing fish oil with vegetable oil sources on the growth performance of salmonids has not been well studied. Virtually all studies that have been reported have replaced fish oil with vegetable oil but left fish meal or other animal byproducts in the diet which contributed significant cholesterol levels in the diets (For example Alexis et al., 1985; Greene and Selivonchick, 1990; Tocher et al., 2003; Bendiksen et al., 2003). Recent work in our lab showed that the growth rate of fish fed all vegetable diets was significantly less than those of fish fed diets where vegetable protein was replaced with a 5% inclusion rate of fish meal (Drew et al., unpublished data). This suggests there is a growth factor or factors present in fish meal. Cholesterol may be one of these factors, however, to our knowledge there has been no controlled experiment which conclusively examines the effect of: 1) replacing fish oil with vegetable oils in diets with no other marine or animal source ingredients or 2) the effect of adding cholesterol to cholesterol free diets on growth performance in salmonid fish 3) the use of tallow and MBM as cholesterol sources in salmonid fish. The following report outlines the results of studies performed to address these 3 objectives.

Methods

Experiment 1

Objective: To determine whether cholesterol is a conditionally required nutrient in rainbow trout and whether the level of omega-3 fatty acids in the diets affects the requirement for cholesterol.

The experiment used 27 x 60 L tanks of rainbow trout with 13 fish per tank at the beginning of the experiment. The initial body weight of the fish was ~ 12 g. The fish were fed 8 diets in a 2 x 4 factorial arrangement with 4 levels of cholesterol (0, 0.05, 0.1 and 0.15% as is basis) and 2 levels of omega-3 fatty acids (linseed oil and olive oil) (Table 1). The diets were formulated to contain 40% digestible crude protein and 18.82 MJ/kg digestible energy. The diets were pelleted and fish were fed to satiation 3 x per day for the 12 week duration of the experiment. Fish were weighed on days 0, 28, 56 and 84 of the experiment and feed intake was measured daily. At the end of the experiment, the fish were killed and 1 mL of blood per fish was collected and livers were removed and frozen in liquid nitrogen for subsequent analysis of cholesterol using a commercial assay kit. The statistical analysis of growth parameters and mortality was done using SPSS. The experiment was analyzed as a 2 x 4 factorial arrangement of treatments in a completely randomized design. When interactions were significant, the experiment was reanalyzed as 8 separate treatments in a completely randomized design. Means were separated using Ryan Einot Gabriel Welsch F test and differences were considered different when $P < 0.05$.

Experiment 2

Objective: To determine whether cholesterol is a conditionally required nutrient in rainbow trout and whether the level of omega-3 fatty acids in the diets affects the requirement for cholesterol.

The experiment used 24 x 360 L tanks of rainbow trout with 20 fish with a mean weight of 28 g at the beginning of the experiment. The fish were fed 6 diets in a 2 x 3 factorial arrangement with 3 levels of cholesterol (0, 0.075 and 0.15% as is basis) and 2 levels of omega-3 fatty acids (linseed oil and olive oil) (Table 3). The diets were formulated to contain 40% digestible crude protein and 18.82 MJ/kg digestible energy. The diets were pelleted and fish were fed to satiation 2 x per day for the 12 week duration of the experiment. Fish were weighed on days 0 and 56 of the experiment and feed intake was measured daily. The statistical analysis of growth parameters was done using SPSS. The experiment was analyzed as a 2 x 3 factorial arrangement of treatments in a completely randomized design. Means were separated using Ryan Einot Gabriel Welsch F test and differences were considered different when $P < 0.05$.

Experiment 3

Objective: To examine the use of tallow and meat and bone meal (MBM) as sources of cholesterol in diets fed to rainbow trout.

Three diets were used in this experiment: a control diet containing no cholesterol, a diet containing 4% tallow replacing canola oil and a diet containing 10% MBM replacing canola oil and plant proteins. The diets were formulated to contain 40% digestible crude protein and 18.82 MJ/kg digestible energy and contained no marine products of any kind. The experiment used 12 x 75 L tanks of rainbow trout with 10 fish with a mean weight of 115 g at the beginning of the experiment. The diets were pelleted and fish were fed to satiation 2 x per day for the 12 week duration of the experiment. Fish were weighed on days 0 and 56 of the experiment and feed intake was measured daily. The statistical analysis of growth parameters was done using SPSS. The experiment was analyzed as a completely randomized design. Means were separated using Ryan Einot Gabriel Welsch F test and differences were considered different when $P < 0.05$.

Results

Experiment 1

The effect of levels of PUFAs and cholesterol on growth and mortality in the fish during the experiment are shown in Table 2. Level of PUFA in the diets had a significant effect on fish growth for total weight gain, feed intake, final body weight, mortality and thermal growth coefficient during the experiment. Fish fed the mixture of linseed and olive oil had significantly higher growth performance and significantly lower mortality levels

during the experiment ($P < 0.05$). The effect of cholesterol on growth performance was only significant in the case of feed intake with fish receiving 0.15% cholesterol eating significantly more than those fed diets with 0% cholesterol. However, there was a trend towards faster growth, final body weight and thermal growth coefficient with P -values ranging from 0.053-0.074. There were also significant interactions between the main effects for feed intake and thermal growth coefficient and near significant interactions for gain and final body weight.

Due to the significant interaction, we reanalyzed the experiment as 8 separate treatments. The interaction was similar for all of the parameters measured with no effect of adding cholesterol in the fish fed the linseed/olive oil diets and a linear response to added cholesterol in the fish fed the olive oil diets. The results suggest that in diets deficient in PUFA's the additional deficiency in cholesterol negatively impacts fish growth performance. This is supported by the interaction of cholesterol and PUFAs on fish mortality. Fish that received low levels of cholesterol combined with olive oil had significantly higher mortality rates than fish fed diets containing linseed oil. Interestingly, mortality increased at the highest level of cholesterol suggesting that the optimal level of cholesterol for rainbow trout is between 0.05 and 0.10%.

Analysis of the diets and ingredients showed that S-type gold fat contained cholesterol in spite of being a plant-origin fat. We therefore reformulated the diets and duplicated the experiment.

Experiment 2

The effect of oil source was not significant in this experiment, indicating that the level of PUFAs in the diet did not affect the requirement of the trout for cholesterol. The effect of cholesterol was significant for ADG, Feed:Gain ratio and specific growth rate ($P < 0.05$). However, feed intake was not significantly affected by the level of cholesterol in the diet. There were no significant interactions between PUFAs and cholesterol level.

The results of this experiment differ from experiment 1, however, it is important to note that the only source of cholesterol in these diets was the synthetic cholesterol. In experiment 1, cholesterol was also added in the S-type gold fat. Thus experiment 1 is probably a better indication of the requirement for dietary cholesterol in rainbow trout. Experiment 2 also used larger fish than experiment 1. Thus the lack of effect of PUFA level on growth may be due to the size and metabolic differences between the fish used in the two experiments.

This experiment conclusively demonstrates that rainbow trout have a dietary requirement for cholesterol to maximize growth. Furthermore, this requirement is greater than 0.075%.

Experiment 3

The fish fed the tallow diet had significantly higher ADGs, feed:gain ratio and specific growth rates than the negative controls ($P < 0.05$). As in Experiment 2 ADFI was not affected by diet. Given that the only differences between the tallow diet and the negative control were the fat source, this result supports the hypothesis that rainbow trout require cholesterol to maximize growth rates. The fish fed the MBM diets grew significantly faster than either the tallow or negative control groups. Given that the diets were nutritionally balanced for amino acids and energy, this indicates the presence of a nutrient or growth factor in MBM in addition to cholesterol. Thus further work will be required to determine the factors present in MBM that might account for this effect.

Conclusions and Recommendations

- 1) Rainbow trout have a requirement for dietary cholesterol to achieve maximum growth rates. The requirement is approximately 0.015% of the diet.
- 2) Diets lacking in cholesterol result in poorer growth rate and feed conversion but do not affect feed intake.
- 3) The use of cholesterol-containing fats such as tallow can meet this requirement for cholesterol.
- 4) The use of rendered protein products such as meat and bone meal can also be used to meet the cholesterol requirement of rainbow trout. Meat and bone meal also appears to contain other growth factors which improve the growth performance of rainbow trout.

Table 1. Diet formulations used in Experiment 1.

[illegible]

Table 2. The effects of polyunsaturated fatty acid level (PUFA) and cholesterol level on fish growth parameters over the entire 12 week period of Experiment 1.

	Total gain (g/tank)	Feed intake (g/tank)	Feed:Gain (g:g)	Final Tank Weight (g)	Mortality (%)
PUFA					
Olive oil	8070	7600	1.08	9390	15.4
Linseed/Olive oil	10700	9330	1.14	12000	1.34
CHOLESTEROL					
0.00	8380	7370a	1.13	9700	16.2
0.05	9010	8310ab	1.10	10300	6.62
0.10	9770	8470ab	1.15	11100	1.28
0.15	10400	9710b	1.06	11700	9.30
Pooled SEM	414.8	353.3	0.0219	418.7	3.328
P-VALUES					
PUFA	<0.010	<0.010	0.130	<0.010	0.033
Cholesterol	0.073	0.001	0.508	0.074	0.389
Interaction	0.055	0.008	0.445	0.053	0.350

Table 3. Diet formulations used in Experiment 2.

	Olive oil			Tallow		
	0	0.075	0.15	0	0.075	0.15
Corn Gluten Meal	160	160	160	160	160	160
Soy Protein Conc.	210	210	210	210	210	210
Wheat Gluten	230	230	230	230	230	230
Wheat Middlings	170	170	170	170	170	170
Canola/Linseed Oil	0	0	0	100	100	100
Olive Oil	170	169.25	168.5	70	69.25	68.5
Cholesterol	0	0.75	1.5	0	0.75	1.5
Vitamin/Mineral Premix	6.45	6.45	6.45	6.45	6.45	6.45
Lysine HCl	20	20	20	20	20	20
DL-Methionine	5	5	5	5	5	5
Taurine	2	2	2	2	2	2
Choline Cl	3.45	3.45	3.45	3.45	3.45	3.45
KH ₂ PO ₄	20	20	20	20	20	20
NaCl	3	3	3	3	3	3
Celite	0.1	0.1	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000	1000	1000

Calculated Analysis	Olive oil			Tallow		
	0	0.075	0.15	0	0.075	0.15
Dig. Dry Matter %	65.65	65.65	65.65	65.65	65.65	65.65
Dig. Protein %	41.07	41.07	41.07	41.07	41.07	41.07
Dig. Energy MJ/kg	18.82	18.82	18.82	18.82	18.82	18.82
Dig Prot.:Energy g/MJ	21.82	21.82	21.82	21.82	21.82	21.82

Table 4. The effects of polyunsaturated fatty acid level (PUFA) and cholesterol level on fish growth parameters over the 8 week period of Experiment 2.

Oil Source	ADG	ADFI	F:G	SGR
Olive Oil	1.09	0.83	0.91	1.65
Linseed Oil	1.05	0.85	0.84	1.69
SE	0.115	0.020	0.043	0.067

Cholesterol				
0	0.89	0.82	0.93	1.56
0.075	0.95	0.86	0.91	1.62
0.15	1.37	0.82	0.77	1.84
SE	0.059	0.038	0.034	0.031

P-values				
PUFA	0.803	0.741	0.549	0.991
Cholesterol	<0.05	0.858	<0.05	<0.05
Interaction	0.917	0.71	0.962	0.985

Table 5. Diet formulations used in Experiment 3.

Ingredient Name	Negative Control	Meat/Bone Meal	Tallow
Canola Oil	129.08	127.66	89.08
Dicalcium Phosphate	39.04	8.55	39.04
DL-methionine	1.02	0	1.02
Soybean Meal	196.85	218.05	196.85
Soy Protein Concentrate	100	61.38	100
Corn Gluten Meal	170	120.36	170
Wheat	150	150	150
Tallow	0	0	40
Cholesterol	0	0	0
Canola Protein Concentrate	200	200	200
Poultry Vit/Min Premix	10	10	10
Choline Cl	4	4	4
Meat and Bone Meal	0	100	0

Calculated Analysis

Nutrient Name	%	%	%
Cholesterol	0	0.039	0.016
Total Phosphorus	1.1	1	1.1
DE Trout (Kcal/kg)	4,200.00	4,200.00	4,200.00
Met+Cys	1.68	1.54	1.68
Crude Protein	38.62	38.62	38.62
Methionine	0.78	0.74	0.78
Cysteine	0.72	0.68	0.72
Met+Cys	1.5	1.33	1.5
Lys	1.82	1.89	1.82
Thr	1.48	1.46	1.48
Arg	2.47	2.56	2.47
Ile	1.72	1.66	1.72
Val	1.98	1.96	1.98

Table 6. The effects of addition of tallow or meat and bone meal fish growth parameters over the 8 week period of Experiment 3.

Treatment	ADG	ADFI	Feed:Gain
Negative Control	1.19a	1.73	1.53a
Tallow	1.70b	2.15	1.27ab
Meat and Bone Meal	2.36c	2.09	0.89b
SEM	0.183	0.090	0.115

Acknowledgments

We have acknowledged the financial support of the Agricultural Development Fund on a poster presented at the European Aquaculture meeting in Istanbul Turkey. A copy of the poster is attached to this report.

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Other

This work will form the research section of a M.Sc. thesis by Nevada Young. This grant supported Ms. Young during this period. A manuscript is in preparation for submission to the journal Aquaculture Nutrition.

Publications

Young, N., M.D. Drew and D.P. Bureau 2007. Cholesterol supplementation in plant-based diets for rainbow trout (*Oncorhynchus mykiss*). European Aquaculture Society Special Publication 37, 616-617.

Presentations

Poster based on the publication above was presented at the European Aquaculture Meeting, October 24-27, 2007 in Istanbul Turkey. The poster is attached below.

Extension activities

We have been invited to present these results at the meeting of the Canada, Norway, US Trilateral Research Group for Finfish Aquaculture this August in Trondheim Norway.

Expense Statement

A final statement will be sent by research services.



CHOLESTEROL SUPPLEMENTATION IN PLANT-BASED DIETS FOR RAINBOW TROUT (*Onchorhynchus mykiss*)

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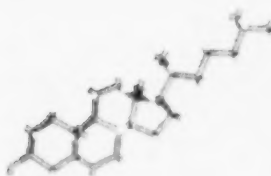
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Introduction

A large amount of research has focused on plant oil and protein as replacements for fish meal and oil. However, the performance of salmonid fish is significantly reduced when fed diets devoid of marine products. Although teleost fish have ability to synthesize cholesterol, the rate of synthesis may be insufficient for maximum growth rates. Polyunsaturated fatty acids (PUFA's) have been shown to suppress expression of rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase. This study was conducted to determine if rainbow trout have a conditional requirement for cholesterol and the effect of dietary PUFA's on this requirement.

Structure of Cholesterol



Materials and Methods

- A 12 week growth trial was conducted at Fish Nutrition Research Laboratory in Guelph, Ontario using 312 (~13 g each) rainbow trout.
- 13 fish were randomly assigned to one of 24 tanks (60 L).
- 8 experimental diets were arranged in 2X4 factorial design with 2 levels of PUFA (olive oil or olive oil + linseed oil 50:50) and 4 levels of cholesterol (0.00, 0.05, 0.10, and 0.15 mg/g).
- Diets contained no animal or marine ingredients and contained 400 g/kg digestible crude protein, 17.14 MJ/kg DE, 111 g/kg lipid and met NRC requirements for all other nutrients.
- Fish were weighed and feed intake was measured on days 0, 28, 56, and 84.
- Experiment was analyzed using SPSS. Means were separated using the RLGWF test and differences considered significant when $P < 0.05$.

Results

- The interaction between PUFA and cholesterol was significant so the experiment was reanalyzed as a completely randomized design.
- Fish fed the olive oil diet had significantly lower growth rates and feed intakes at the 0 cholesterol level compared to those fed olive + linseed oil.
- Using regression analysis, it was determined that addition of cholesterol significantly increased feed intake and gain in fish fed olive oil diets, but not those fed olive + linseed oil diets.
- There was no significant effect of treatment on feed gain ratios.
- Fish fed the olive oil diets had significantly higher levels of mortality at the 0 and 0.05 mg/g level of cholesterol compared with those fed olive + linseed oil. Mortality was due to bacterial cold water disease.

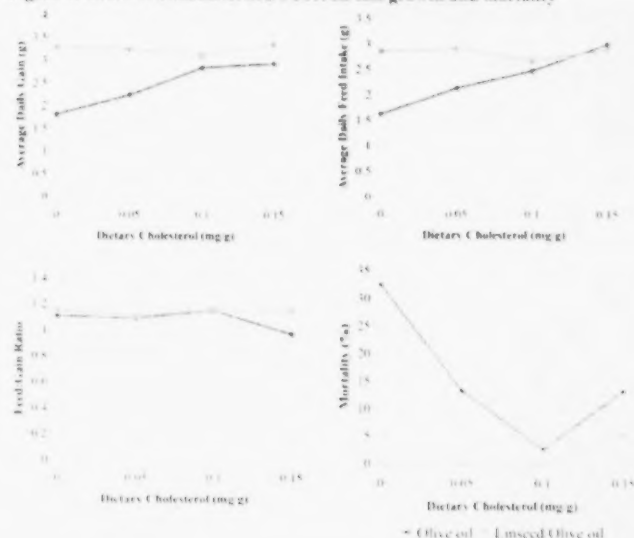
Conclusions

- Cholesterol had a significant effect on growth in rainbow trout fed a low PUFA diet.
- Olive oil contains no *n-3* fatty acids (linolenic), which fish cannot synthesize *de novo*. This may have resulted in a deficiency of these fatty acids.
- The dramatic effect of cholesterol supplementation observed in low PUFA diets may be explained by the more important biological functioning of the cholesterol phospholipid ratio in fish, as opposed to the activity of the HMG-CoA reductase enzyme in cholesterol synthesis.

Acknowledgements

The authors acknowledge the financial support provided by the Saskatchewan Agricultural Development Fund.

Figure 1. Effect of cholesterol and PUFA on fish growth and mortality



^{ab} Means with different labels are significantly different ($P < 0.05$)

